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ORIGINAL ARTICLE **OPEN ACCESS**

Antimicrobial activity of leaf extracts of *Mansoa alliacea* (Lam.) A. H. Gentry

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ABSTRACT
Antimicrobial efficiency of Mansoa alliacae (Lam.) A. H. Gentry leaf extracts against Listeria monocytogenes, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Candida albicans and Fusarium oxysporum were evaluated by the agar well diffusion method. Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) of leaf extracts were determined against selected bacterical and fungal cultures. Among the four bacterial strains tested, petroleum ether (MPE) leaf extract showed maximum zone of inhibition for K. pneumoniae(18 mm), chloroform (MC) leaf extract inhibited S. aureus (13 mm) only, ethyl actetate (MEA) leaf extract effectively inhibited the growth of E. coli (16 mm), ethnolic (ME) leaf extract inhibited K. pneumoniae (12 mm) and aqueous (MW) leaf extract showed best results against L. monocytogenes (13 mm). Except for MW all other leaf extracts showed an inhibitory effect against Fusarium oxysporum, but none of the extracts were able to inhibit the growth of Candida albicans. The MIC values for leaf extracts against selected pathogens was 200 mg/ml, the MBC and MFC values were 200 mg/ml to > 200 mg/ml. The present study revealed that M. alliacae leaf extracts exhibited effective inhibitory activity against the tested microbes.
Keywords: Antimicrobial, Mansoa alliacea, chloramphenicol, MIC, MBC, MFC

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INTRODUCTION

Microorganisms include bacteria, actinomycetes, yeasts, molds, and viruses, among which bacteria are the most prevalent, accounting for 90%-95% of microorganisms. Microorganisms are tiny and closely related to humans, comprised of a variety of beneficial and harmful species. According to CDC's AR threats report (2019), more than 2.8 million antibiotic-resistance infections occurs in the U.S every year and more than 35,000 people die as a result of it [1]. The WHO estimates 25% of the total 57 million annual deaths that occur worldwide are caused by microbes and this population is significantly higher in developing countries[2].The number of multi-drug resistant microbial strains and evolution of reduced susceptibility to antibiotics are continuously increasing. The problem of antibiotic resistance is a global concern. Till today, there are no available resources to reverse antibiotic resistance in bacteria. The discovery and development of Penicillin in the 1990s gave hope to medical sciences, but today bacteria and other microorganisms have become resistant to antibiotics to adapt and evolve. The evolution of new resistant bacterial strains which are more lethal than their parental strains is attributed to the use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. According to the reports of WHO, resistance was more efficient in bacterial infections which cause many deadly diseases like diarrhoea, meningitis, syphilis, gonorrhoea and tuberculosis. *Staphylococcus aureus, Streptococcus pyogenes*and *Mycobacterium tuberculosis*have become resistant to more drugs and are considered as multiple drug-resistant microbes. In many developing countries, synthetic drugs are not only expensive and inadequate for the treatments but also with adulterations and many side effects. To overcome the side effects and problems prone to antibiotics, presently, many research workers are looking for novel antimicrobial agents which has a broad spectrum of activity against both Gram-positive and gram-negative bacteria. These researchers are keener in exploring different medicinal plants described in Ayurveda, Sushrut Samhita, Charak Samhita and other available kinds of literature[3,4].Among 17,000 discovered plant species, about 3,000 species are used in the medicinal field. About 80% of individuals from developed countriesuse traditional medicines, which has compounds from medicinal plants, hence such plants are investigated for a better understanding of their properties, safety and efficacy [5]. Antimicrobial properties in plants are attributed to the presence

of active compounds like quinones, phenols, alkaloids, flavonoids, terpenoids, essential oil, tannins, lignans, glucosinolates and some secondary metabolites. Scientific validation of traditional health care systems prevalent in tribal societies, ethnobotanical literature and plants described in Ayurveda, using modern analytical tolls is currently an active area of research [3].

The present study is aimed at evaluating the antimicrobial efficacy of *Mansoa alliacea* leaf extracts against two gram-positive, two gram-negative pathogenic bacterial strains and two pathogenic fungal strains.

MATERIAL AND METHODS

Collection and authentication of plant

M. alliacea plant was collected from Basavanagudi, Bengaluru, Karnataka. The plant was identified and authenticated by the taxonomists at Botanical Survey of India (BSI), Pune and the voucher specimen of reference no. BSI/WRC/IDEN.CER/2018.H2/55 was deposited with Accession/voucher No.:136264

Preparation of leaf extracts by Soxhlet extraction

The collected fresh leaves of *M. alliacea* were washed under running tap water, shade dried and then blended to a fine powder using a mechanical blender. The fine particles were sieved and stored in clean containers. Petroleum ether (MPE), chloroform (MC), ethyl acetate (MEA), ethanolic (ME) and aqueous (MW) leaf extracts were prepared using the soxhlet extraction method by adding 200 ml solvent to 10 gm of the leaf powder taken in Whatman cellulose timble for about 24 hours or till the solvent become colourless. The liquid extract obtained was concentrated using a rotary evaporator (Superfit RotaVap model: PBV – 7D) stored at 4˚C for further use.

Bacterial and fungal cultures

Pure cultures of bacteria and fungi *viz., Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 118), *Klebsiella pneumonia*(MTCC 109), *Candida albicans*(MTCC 183) and *Fusarium oxysporum* (MTCC 1755) were procured from the Microbial Type Culture Collection and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. *Listeria monocytogenes* (Scott A) was procured from CFTRI, Mysuru. The bacterial cultures were maintained on Brain heart infusion (BHI) media and the fungal cultures on the potato dextrose broth at 30- 37^{o} C. Each bacterial and fungal culture were further maintained by subculturing regularly on the same media and stored at 4^{o}C until further use.

Determination of zone of Inhibition by agar well diffusion method

Antibacterial and antifungal activities of *M. alliacea* leaf extracts were evaluated by the agar well diffusion method [6,7,8].BHI agar (1.5% w/v agar) pre-inoculated with the bacterial pathogen (1% v/v) and PDA agar pre-inoculated with fungal pathogen were poured into sterile petri-dish and allowed to solidify. Wells of 4 mm were bored using sterile cork borer and 100 µL of different leaf extracts were added (100 mg/ml). The petri plates were kept for 30 min at 4°C for proper diffusion, incubated at 37°C for 24 h and observed for the zone of inhibition (mm in diameter). Chloramphenicol (1 mg/ml) andNystatin (1 mg/ml) were used as positive controls for bacterial and fungal cultures, respectively.

Leaf extracts showing a better zone of inhibition against bacterial and fungal strains were selected for the determination of Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC).

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations are considered the gold standard for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing [9,10]. MIC of ***M. alliacea*** leaf extracts against selected bacterial and fungal pathogens was performed using the broth dilution method. Fungal spore (1 X 10^{6} spores/ml) was suspended in PDB broth and overnight broth cultures of bacteria was suspended in BHI broth with turbidity adjusted to 0.5 McFarland, resulting in a suspension containing approximately10^{8} CFU/ml. To measure the MIC, 100 µl of respective broth was poured into 8 wells of a 96-wellmicrotiter plate. In the first well, 100 µl of the leaf extract was added. A two-fold dilution was then made to obtain a different concentration in each well. Then, 100 µl of the microbial suspension was added to each well of microplate and incubated at 37°C for 24 h (for bacterial strains), 30°C for 2 days (for fungal strain). The sample concentration in the well without visible growth of the bacterial and fungal cells was considered as MIC value. A positive control containing medium with the pathogen and a negative control containing only broth were used for the comparison. This was performed in triplicates to confirm its value for the tested organisms.

Determination of MBC (Minimum bactericidal concentration)

To determine the MBC, 100 µl of broth was taken from all wells of MIC plate that showed no visible signs of growth/turbidity (MIC and higher dilutions) and spread on respective agar plates [10]. The plates were then incubated at 37°C for 24-48 h. The MBC is the least concentration of samples that prevented the growth of the bacteria.

Determination of MFC (Minimum fungicidal concentration)

100 µl of broth was taken from all wells of MIC plate that do not have visible signs of growth/turbidity (MIC and higher dilutions) and spread on respective agar plates [11]. The plates were then incubated at 30°C for 2-3 days. Later, the concentration of samples which completely prevented the growth of the fungi was considered as MFC.

Determination of IC50 concentration

Different concentrations (3.12 mg – 200 mg) of ***M. alliacea*** leaf extraction was taken in the different wells of 96 well plates to determine the IC 50 values. The volume was madeup to 100 µl using respective broth. Later, 100 µl of pathogen broth suspension was added and the plates were incubated at 37°C for 24 h (for bacterial strains), 30°C for 2 days (for fungal strains). Positive control with the pathogen, but without extract and negative control was only broth was taken. Chloramphenicol and Nystatinwere used as standard. After incubation, absorbance was read at 600 nm. IC50 value is the concentration of the sample required for inhibiting 50% of the pathogenic strain.

Because of the coloured samples OD reading at 600 nm was taken and substrated with sample control and percentage reduction in absorbance was calculated compared to control.

RESULTS AND DISCUSSION

Determination of zone of Inhibition by agar welldiffusion method

Antimicrobial activity of ***M. alliacea*** leaf extract was tested by agar well diffusion method according to their zone of inhibition against four bacterial (two gram +ve and two gram -ve) and two fungal pathogens and the results were compared with the positive controls (chloramphenicol for bacteria and nystatin for fungi). The results showed different antimicrobial activity against the bacterial (Fig. 1 (a-d)) and fungal strains (Fig. 2 (e-f)). Among four bacterial strains tested, petroleum leaf extract (MPE) was able to inhibit the growth of *S. aureus* and *K. pneumoniea* only and showed maximum inhibition zone with a diameter of 18 mm for*K. pneumoniea*, and 15 mm in diameter for *S. aureus*. The chloroform leaf extract (MC) showed inhibitory activity against *S. aureus* only with the zone of inhibition of 13 mm in diameter. Except for*S. aureus*, the growth of all other tested bacteria were inhibited by ethyl acetate leaf extract (MEA) showing a zone of inhibition of 10 mm (against *L. monocytogenes* and *K. pneumoniea*) and 16 mm (against *E. coli*). *S. aureus* and *E. coli* were resistant against ethanolic leaf extract (ME), whereas, *L. monocytogenes* (11 mm) and *K. pneumoniea* (12 mm)were susceptible to treatment with ME. Aqueous leaf extract (MW) proved to be potent antibacterial against all the bacterial strains except *E.coli*. The inhibition zone for *L. monocytogenes* was measured to be 13 mm and 11 mm in diameterfor *S. aureus* and *K. pneumoniea* (Table 1).

Except for MW all other leaf extracts were able to inhibit the growth of *Fusarium oxysporum* with its zone of inhibition nearly the same as that of standard nystatin (18 mm) (Table 1). But none of the extracts was able to inhibit the growth of *Candida albicans.*

Based on these results, minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) were determined using the leaf extracts which are effectively inhibited the growth of the bacterial and fungal strains with the best zone of inhibition.

Determination of MIC, MBC and MFC

MIC of *M. alliacea* leaf extracts against selected bacterial and fungal cultures were determined by the broth dilution method. The least concentration of the plant extracts that were able to inhibit the growth of microorganisms completely (absence of turbidity) after the incubation periodwas recorded as the MIC value of that extract. The MIC was found to be 200 mg/ml for all the leaf extracts against the tested pathogens based on the level of turbidity in each well of 96 well plate (Table 2 &3).

The minimum bactericidal concentration of ME and MW against *L. monocytogens* was found to be 200 mg/ml. MBC for MPE against *K. pneumoniae* was >200 mg/ml. MC showed MBC of >200 mg/ml against *S. aureus* and MEA had MBC of 200 mg/ml against *E. coli*(Table 2).

MPE, MC and ME leaf extracts showed MFC of >200 mg/ml, but MEA proved to be a better antifungal extract against *F. oxysporum* with an MFC value of 200 mg/ml (Table 3).

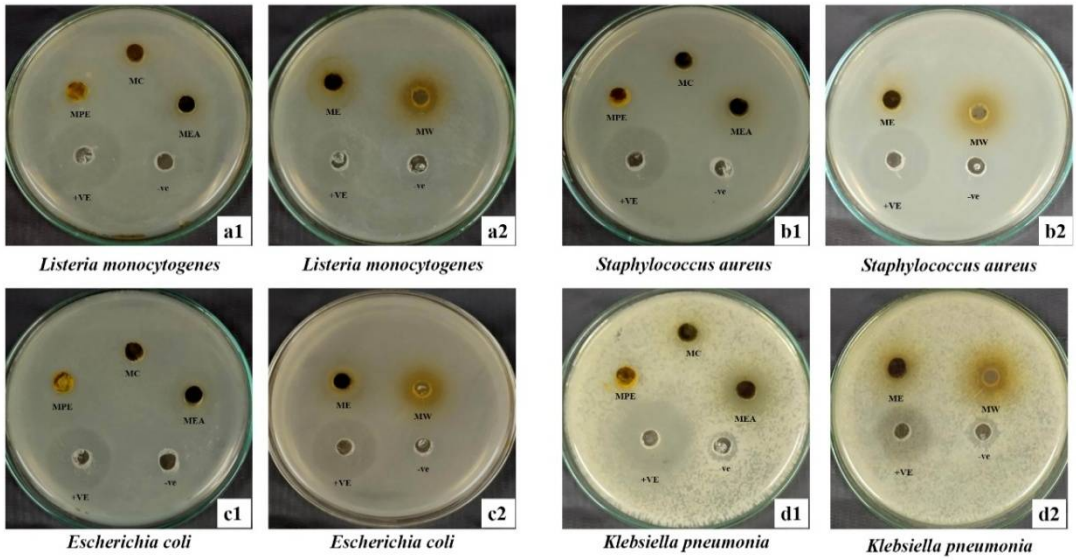


*Figure 1*Antibacterial activity - Plates showing zone of inhibition in mm diameter of petroleum leaf extracts (MPE), chloroform leaf extract (MC), ethyl acetate leaf extract (MEA), ethanolic leaf extract (ME), aqueous leaf extract (MW), chloramphenicol (+ve control) and -ve control

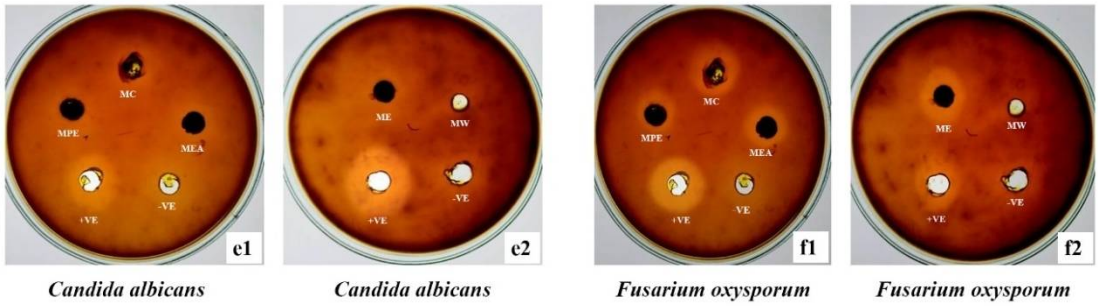


Figure 2: Antifungal activity - Plates showing zone of inhibition in mm diameter of petroleum leaf extracts (MPE), chloroform leaf extract (MC), ethyl acetate leaf extract (MEA), ethanolic leaf extract (ME), aqueous leaf extract (MW), chloramphenicol (+ve control) and –ve control

Sl.No.	Sample	Inhibition zone (mm in diameter)					
		Listeria monocytogenes	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Escherichia coli***	***Candida albicans***	***Fusarium oxysporum***
1	MPE	-	15	18	-	-	15
2	MC	-	13	-	-	-	19
3	MEA	10	-	10	16	-	18
4	ME	11	-	12	-	-	15
5	MW	13	11	11	-	-	-
6	Antibiotic	24s	24	25	27	18	18

Table 1: Diameter of zone of inhibition (in mm) of MPE (petroleum ether leaf extract), MC (chloroform leaf extract), MEA (ethyl acetate leaf extract), ME (ethanolic leaf extract), MW (aqueous leaf extract) and antibiotic (chloramphenicol)

Sl. No.	Sample	Organisms	MIC (mg/ml)	MBC (mg/ml)
1	**MPE**	*K. pneumoniae*	200	>200
2	**MC**	*S. aureus*	200	>200
3	**MEA**	*E. coli*	200	200
4	**ME**	*L. monocytogens*	200	200
5	**MW**	*L. monocytogens*	200	200

Table 2: MIC and MBC of *Mansoa alliacea* leaf extracts against bacterial cultures.MPE (petroleum ether leaf extract), MC (chloroform leaf extract), MEA (ethyl acetate leaf extract), ME (ethanolic leaf extract), MW (aqueous leaf extract)

Sl. No.	Sample	Organism	MIC (mg/ml)	MFC (mg/ml)
1	**MPE**	*F. oxysporum*	200	>200
2	**MC**	*F. oxysporum*	200	>200
3	**MEA**	*F. oxysporum*	200	200
4	**ME**	*F. oxysporum*	200	>200

Table 3: MFC of *Mansoa alliacea* leaf extracts against *Fusarium oxysporum.* MPE (petroleum ether leaf extract), MC (chloroform leaf extract), MEA (ethyl acetate leaf extract) and ME (ethanolic leaf extract)

Determination of IC50 concentrations

Based on the MIC, MBC and MFC values IC 50 of *M. alliacea* leaf extracts was calculated by treating the pathogens with different concentrations (3.12 mg – 200 mg) of the extracts. The percentage of bacterial (Fig 3) and fungal (Fig 5) growth gradually decreased with the increase in concentration. IC 50 value for selected microorganisms were calculated using a linear regression curve in MS Excel 2016. The IC 50 value of MPE against *K. pneumoniae* was 37.5 mg/ml, MC against *S. aureus* was 31.2 mg/ml and MEA against *E. coli* was 25 mg/ml. MW showed better antibacterial activity against *L. monocytogenes* with an IC 50 value of 12.5 mg/ml than the ME (IC 50 – 18.75 mg/ml) (Fig 4).

Among four different leaf extracts tested for antifungal efficacy against *F. oxysporum,*MC and MEA showed the best inhibitory effect with an IC value of 18.75 mg/ml, whereas MPE and ME showed IC 50 value of 25 mg/ml (Fig 6).

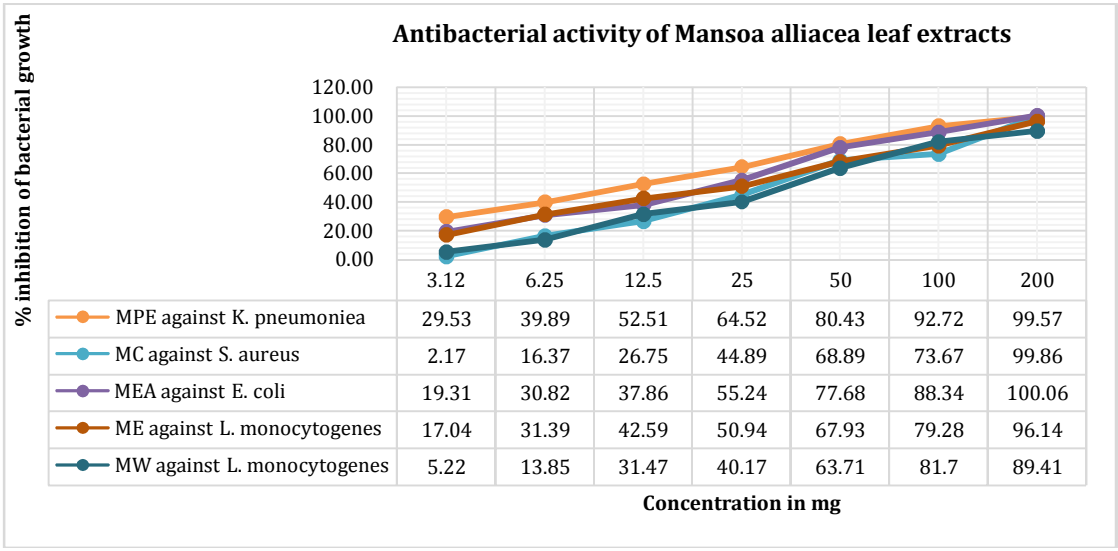


Figure 3: % inhibition of bacterial growth by *Mansoa alliacea* leaf extracts at different concentrations

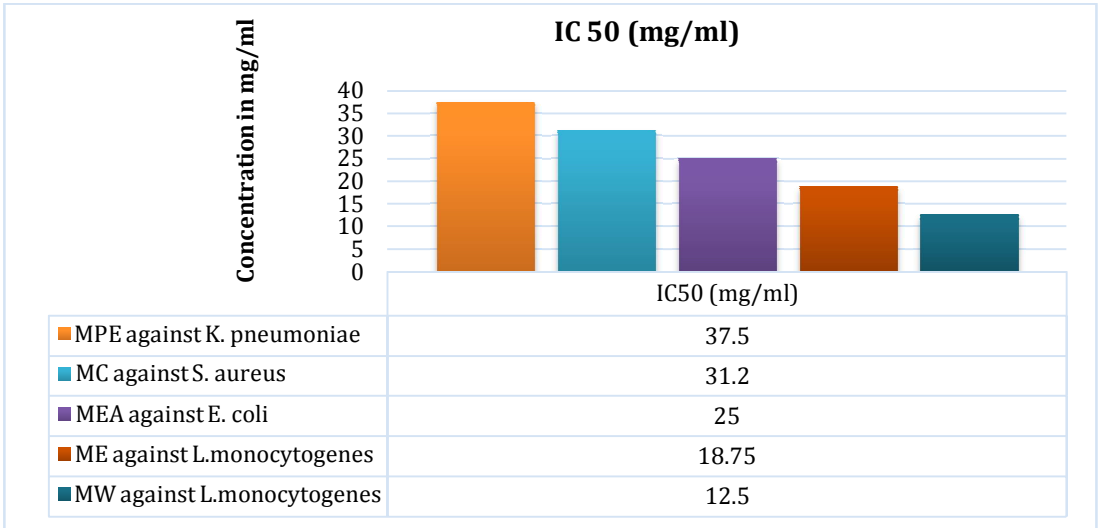
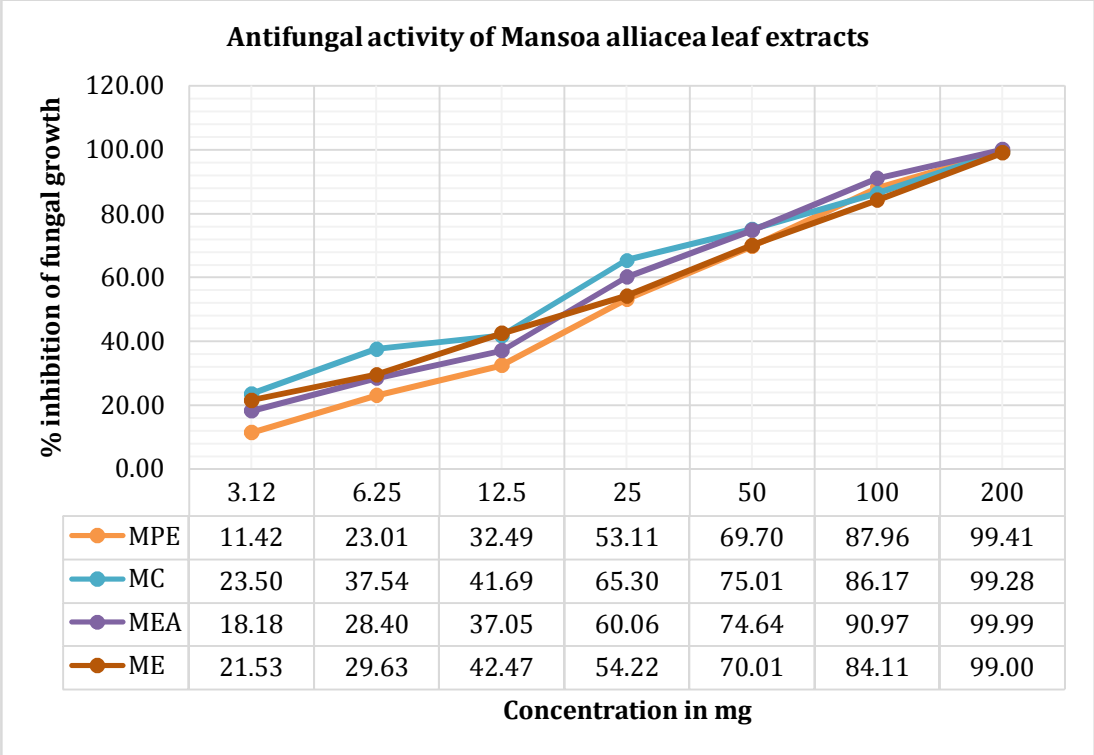
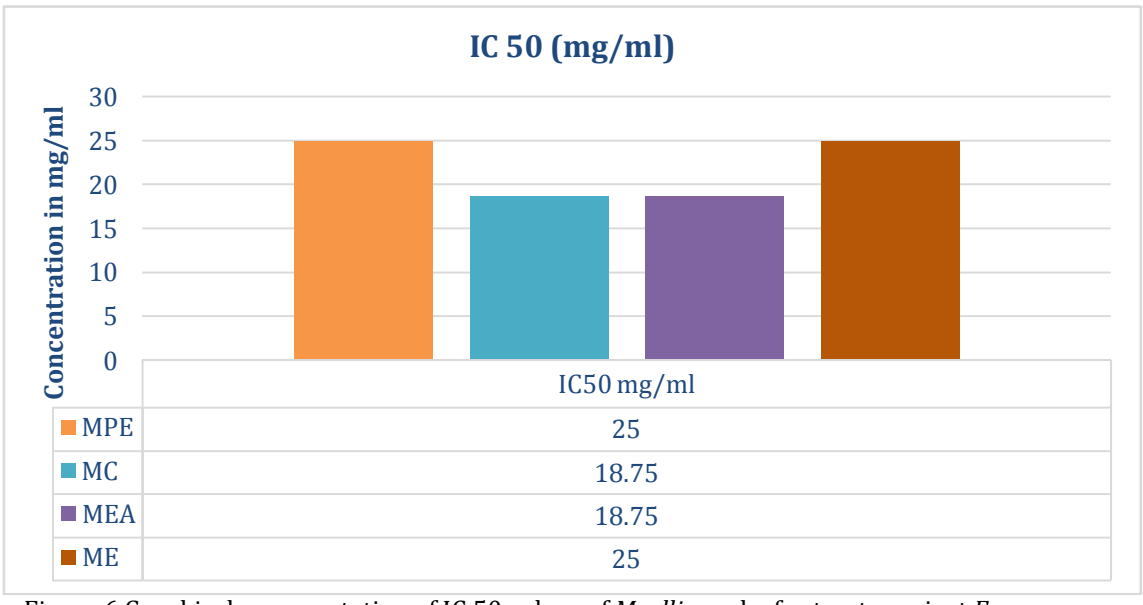


Figure 4: Graphical representation of IC 50 values of *M. alliacea* leaf extracts against selected bacterial strains



	3.12	6.25	12.5	25	50	100	200
MPE	11.42	23.01	32.49	53.11	69.70	87.96	99.41
MC	23.50	37.54	41.69	65.30	75.01	86.17	99.28
MEA	18.18	28.40	37.05	60.06	74.64	90.97	99.99
ME	21.53	29.63	42.47	54.22	70.01	84.11	99.00

Figure 5% inhibition of fungal growth (*F. oxysporum*) by *Mansoa alliacea* leaf extracts at different concentrations



	IC50 mg/ml
MPE	25
MC	18.75
MEA	18.75
ME	25

Figure 6 Graphical representation of IC 50 values of *M. alliacea* leaf extracts against *F. oxysporum*

Sharma (1993) extracted and isolated *n*-alkanes, *n*-alkanols, 16-hentriacontanone and sterols from the petroleum ether leaf extracts of *Adenocalymma alliceum, Annona squamosa* and *Amaranthus ticolor.* He evaluated the antibacterial activity of these active compounds against *S. aureus, S. albus, Steptocorus viridians, E. coli, Pseudomonas pyocyanea* and *Klebsiella* and based on the zone of inhibition he reported that 16-hentriacontanone and sterols exhibited effective antibacterial properties than the *n*-alkanes with moderate response [12]. A simple and rapid method was reported by Rana *et al.,* (1996) to detect the fungi-toxic substance in petroleum ether leaf extract of *Adenocalymma alliceum* against *Alternaria brassicae* and *Aspergillus niger.* A clear zone of growth inhibition was exhibited against the tested fungal strains and they also mentioned that the area of growth inhibition is directly related to the quantity of testing material/ sample, increased concentrations of the extracts resulted in an increased area of growth

inhibition [13]. Antifungal activity of 19 Latin American plants, including *Mansoa alliacea* was screened by Freixa *et al.,* (1998) using agar disk diffusion assay against several fungi. Among all the fungi tested *M. alliacea* leaf extracts showed activity against *Microsporum gypseum* and *Tricophyton mentagrophytes* only [14]. Rana *et al.,* (1999) tested the antifungal activity of aqueous extract of leaves of *A. alliceum* against many fungi. The extract showed broad fungi toxic spectrum as tested by inhibition of spore germination. The inhibition of spore germination was related to the time of exposure and temperature of the extract. They found that, on exposing the *A. brassicae* sporesto the extract for 5 minutes, 72% of inhibition in spore germination was noticed and after exposure for 10 minutes 100% inhibition was found. And the inhibitory activity decreases by increasing the temperature of the extract [15].*A. alliceum* plant extract showed higher levels of antimicrobial activity against *Alternaria alternate, Aspergillus flavus, F. oxysporum, Phizoctonia solani* and *Xanthomonas compestries* with best zone of inhibition [16]. Guilon *et al.,* (2012) analysed the essential oil from the leaves of *Mansoa difficilis* by GCMS and idenfied oct-1-en 3-ol as the major compound present. They tested the hexane and methanol leaf extracts for antimicrobial activity against ten microorganisms and reported that the hexane extract was effective against *Pseudomonas aeruginosa* and *S. aureus*[17]. The leaf extracts of *M. alliacea, Tecomaria capensis* and *Tecoma stans*of Bignoniaceaewere evaluated for their antibacterial potential against two gram +ve bacteria *Bacillus subtilis*, *S. aureus* and two gram –ve bacteria *E. coli* and *P. aeruginosa* using agar well diffusion method by Iltaf *et al.,* (2016). The zone of inhibition was compared with different standards like ampicillin and amikacin. The aqueous leaf extract showed better antibacterial activity with MIC ranging from 10 – 2.5 mg/ml [18].The results of the present investigation are in confirmation of the reported antimicrobial activities by other researchers.

CONCLUSION

The present investigation revealed that the *M. alliacea* leaf extracts exhibited effective inhibitory activity against the tested bacterial and fungal cultures. All leaf extracts except MC showed the best zone of inhibition against *K. pneumoniae*, the growth of *L. monocytogenes* was inhibited by MEA, ME and MW, *S. aureus* by MPE, MC and MW, but *E. coli* was susceptible to MEA only. *C. albicans* was resistant against all the samples, whereas, *F. oxysporum* growth was inhibited by all the extracts except MW. This study gives a good insightinto the antimicrobial efficacy of the selected plant and can be used to treat the infections caused by tested microorganisms.

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